

Variation in allergen content in sublingual allergen immunotherapy with house dust mites

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Keywords

allergen content; efficacy; evidence-based medicine; house dust mites' sublingual allergen immunotherapy; weekly dosage.

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Abstract

Background: Allergen immunotherapy is a treatment modality which can be applied using different vaccines. The aim of this study was to quantify and compare the allergen content of different house dust mites (HDM) sublingual treatments and to review the evidence on their efficacy.

Methods: Five sublingual allergen immunotherapy (SLIT) products were ordered and purchased at an ordinary pharmacy and masked for blinding before the study was started. Detection of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* allergens Der p 1, Der f 1, Der p 2 and Der f 2 was carried out by immunoblotting and fluorescent multiplex. A literature search for meta-analyses and systematic reviews that included SLIT-HDM products was performed.

Results: Der p 1 concentrations ranged from 0.6 to 14.5 µg/ml; similar figures were found for Der f 1 that ranged from 0.2 to 12.4 µg/ml. Der p 2+ Der f 2 ranged from 0.2 to 1.5 µg/ml. Data on efficacy are scarce for most of the five products.

Conclusions: Substantial variations regarding allergen content were found among these five SLIT-HDM products. Therefore, it can be necessary to guarantee the quality of the SLIT-HDM products and to demonstrate their effectiveness before they are marketed. It seems necessary, for the moment, to take into account these characteristics of the products before prescribing.

In the last decade, the gradual development of specific immunological and molecular biology techniques has allowed us to reach a more accurate diagnosis through the identification of allergenic components (molecular diagnosis) (1–4) and it has also helped to develop AIT treatments with a precise allergen content and, therefore, more effective and safer. Beyond the progress in diagnosis, the publication of European guidelines (5) defining the methodology for clinical trials has allowed investigators to better demonstrate the value of the AIT supported by evidence-based medicine (6). Currently, 20 meta-analyses assessing effectiveness of AIT in various indications (asthma and rhinitis), by different administration routes and based on different allergens are available, but only a few products available for prescription are represented in existing meta-analyses (7). Then, an allergist is not sure whether the products available to prescribe are effective (8, 9).

Regarding SLIT-HDM allergens, both the dosages recommended by the different manufacturers in Europe (10) and the composition and quantification of the allergens (11) are diverse. Extracts are complex mixtures of biological materials, and each manufacturer uses a different reference unit (in-house reference standard) to provide batch to batch consistency.

Knowing the allergen content of each therapeutic product is a critical point; however, a fully standardized assessment of allergens is not yet available in Europe despite the efforts of the CREATE project (12). Only some specific techniques and materials are recognized by such project as candidates to be used as standards (13).

We set out to (i) measure the protein composition and to quantify the major allergens in SLIT-HDM products available for prescription in Spain, using the same measurement methods; (ii) identify, by means of a systematic review of the literature, the existing data on the allergen content and the

biological activity of products included; and (iii) review the scientific evidence on their effectiveness.

Methods

In vitro analysis

A comparative study of the five most relevant SLIT-HDM products in Spain (produced under Good Manufacturing Practices conditions) was carried out (in alphabetical order: Allergovac Sublingual Plus, Bial-Aristegui; SLITone ULTRA, ALK-Abello; Staloral 300 Rapid, Stallergenes; Sublivac, Hal Allergy; TOL Forte, Laboratorios Leti). All of them were ordered and purchased at an ordinary pharmacy in Cádiz (Spain), and kept in refrigerator under the same conditions in which patients undergo treatment. Each extract was masked for blinding and a number (from 1 to 5) was randomly assigned to each one before the study was started, so the investigators were not aware, at any time of the study, of which product they were testing. All analyses were performed at the Research and Development Unit, Lobaton Clinic, S.L.P., Cádiz, Spain.

Details on vaccines manufacturing, composition and excipients are shown in Table 1. Excipient concentrations of each extract are not available. To remove all possible excipients, each of the extracts was treated with Vivaspin® 3.0K (Sartorius, Hannover, Germany). Initially, the membranes were washed with deionized water (Milli-Q water, HPLC grade, 18 MΩ from a A10-Synthesis water polishing system, Merck Milipore (Merck KGaA, Darmstadt, Germany)). About 15 ml of Milli-Q water was added to each 5 ml of extract, followed by a centrifugation according to the instructions of the manufacturer. In this way, glycerol and other salts were removed. Centrifugation time needed to reach initial sample volume was used. After treatment with Vivaspin® 3.0K, total protein levels measured by bicinchoninic acid (BCA) were consistent with those found in SDS-PAGE gels. The removal of glycerol and other excipients was needed to avoid interferences with the measurements when taking the same volume for different extracts, as it has been stated by other authors (14) and in the datasheet of the BCA method. However, an analysis was performed with the same method without the use of Vivaspin® 3.0K to evaluate the homogeneity of the results without the removal of glycerol (see Appendix S1). Samples were homogenized by pipetting before loading the sample to gel.

Two different batches of HDM-SLIT maintenance products, all of them consisting of a mix 1 : 1 *D. pteronyssinus*: *D. farinae*, were used (see Table 1).

SDS-PAGE and protein estimation

Equal volumes of each *D. pteronyssinus*: *D. farinae* extract (15 µl) after treatment with Vivaspin® 3.0K were applied to 14% SDS-PAGE in reducing conditions and proteins were visualized by silver staining (15). Electrophoresis was running in a Mini-Protean System (Bio-Rad Laboratories, S.A. (Madrid, Spain)) to 90 V during 1.5 h. Protein content of the extracts was measured using Pierce® BCA protein assay kit (Thermo Scientific) (16).

Table 1 Composition, additives and manufacturing details of vaccines tested

Extracts	Product name – Biological potency	Marketing laboratory	First Batch			Second Batch			Additives
			Reference	Manufacture date	Expiration date	Reference date	Manufacture date	Expiration date	
1	Staloral 300 Rapid – 300 IR/ml	Stallergenes	0001085047	02/2013	02/2014	000111523	07/2013	07/2014	Sodium chloride, glycerol, purified water
2	SLITone ULTRA – 300 SRU	Alk-Abello	027016251001	02/2013	09/2013	027191671001	09/2013	06/2014	Sodium chloride, glycerol, purified water, sodium bicarbonate
3	TOL Forte – 30 HEPL/ml	Laboratorios Leti	2369041100	No data	11/2013	2481042100	No data	02/2014	Sodium chloride, phenol, sodium phosphate monobasic dihydrate, sodium phosphate dibasic dodecahydrate, glycerol, purified water
4	Allergovac Sublingual Plus – 223 432 SU/ml	Bial-Aristegui	ESX0181074	02/2013	02/2014	ESB0673682	09/2013	09/2014	Sodium chloride, glycerol, phenol, purified water
5	Sublivac – 10 000 AU/ml	Hal Allergy	059779	02/2013	12/2013	064456	09/2013	09/2014	Sodium chloride, glycerol, phenol, purified water

Quantification of Der p 1, Der f 1 and group 2 of Dermatophagoides by fluorescent multiplex array (Bioplex)

Equal volumes of each *D. pteronyssinus*/*D. farinae* extract were separated by 14% SDS-PAGE and blotted onto nitrocellulose (Protan[®] Whatman, Dassel, Germany). Aliquots from each extract were diluted 1/500 in assay buffer (PBS, 1% BSA, 0.02% Tween-20, pH 7.4) after treatment with Vivaspin[®] 3.0K, as reported for SDS-PAGE. Again, an analysis with the same methodology without the use of Vivaspin[®] 3.0K was performed to evaluate the heterogeneity of the results (coefficient of variation) without the removal of glycerol (see Appendix S1). Quantification of the allergens was performed by quantitative MARIA[®] assay according to the manufacturer's instructions (Indoor Biotechnologies, Cardiff, UK). Results were expressed in µg/ml after taking into account the dilution factor. The 12-point standard curve ranges were as follows: 125–0.06 ng/ml for Der p1 and Der f1 and 50–0.02 ng/ml for Mite group 2 (Der p2+ Der f2). Standard curves were prepared according to the manufacturer instructions using control standard vial provided with the kit.

ProteOn XPR36 (Plasmon Resonance Interaction System) of Dermatophagoides pteronyssinus/Dermatophagoides farinae extracts

Immobilization of selected capture agent, anti-mouse IgG whole molecule antibody, on Proteon XPR36 GLM chip.

The immobilization step was performed in the horizontal orientation of the ProteOn XPR36 system using a flow rate of 30 µl/min at 25°C on a GLM chip (see Appendix S1). HDM major allergens were screened for with Proteon XPR36.

All binding measurements were taken with PBST as the continuous running buffer at 25°C, following the directions of Bronner et al. (17) with some modifications. In summary, in all experiments, 50 µg of monoclonal antibody was diluted with PBST and injected in the vertical orientation. ProteOn XPR36 fluidics was used for 6 min (150 µl) at 25 µl/min, allowing the mAbs to be captured by the capture agent on the GLM chip (see Appendix S1).

Data processing and analysis

All binding sensograms were collected, processed and analysed using the integrated ProteOn Manager software (Bio-Rad Laboratories) using Langmuir with drift conditions.

Literature review

The identification of the studies in which the content or the biological potency of the SLIT-HDM products was reported was based on a literature research in PubMed, Web of Science and The Cochrane Library databases looking for meta-analyses on SLIT, and the search was limited to the English language but not by publication date. The basic search terms were ('systematic review' OR 'meta-analysis') AND

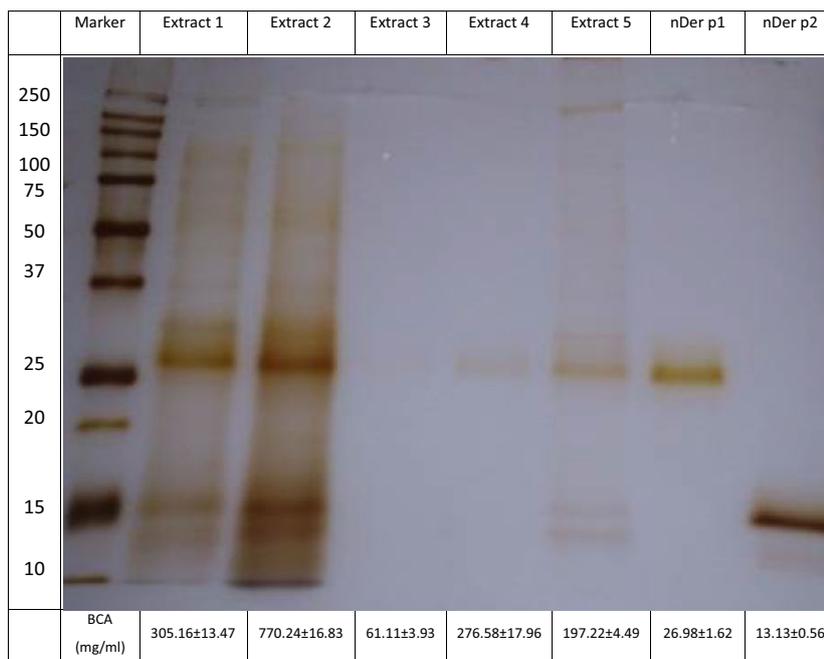


Figure 1 SDS-PAGE (silver nitrate[®] staining is shown): Samples were loaded in sequence (blinded order), 1: Staloral Rapid 300; 2: SLITone ULTRA; 3: TOL Forte; 4: Sublingual Allergovac Plus; 5: Sublivac, with 6 and 7 being native controls for Der p 1 and Der p 2, respectively. M = marker for molecular weight between 250 and 10 kDa. First lane corresponds to molecular weight

marker, followed by extracts 1–5. Lanes 7 and 8 correspond to native Der p1 and Der p2 extracts. Bottom figures show bicinchoninic acid-measured protein levels for each extract (mg/ml). No significant differences were observed between the two studied batches of each extract in the SDS-PAGE gels. (Data not shown).

('sublingual AND immunotherapy') with their corresponding synonyms, MeSH terms and truncations. All the reports that included systematic reviews were selected for full-text reading to gather data on composition and biological potency.

Results

SDS-PAGE and BCA method

Clear-cut differences in the definition of the bands can be seen for the five extracts in SDS-PAGE gels (Fig. 1). A higher intensity is observed in the band of molecular weight (MW) 25 and 14 kDa in the products 1 and 2 (products are listed herein in the order of blinding).

Total protein content measurement was based on the BCA method (see Appendix S1).

Detection of HDM major allergens by immunoblotting

As it can be seen in Fig. 2, after the development with anti-Der p 1 or anti-Der f 1, product 1 had the highest band intensity, followed by product 2; the other products had a

very weak intensity with the volume used. Using a development with anti-Der p 2+ anti-Der f 2, a band can be seen at a MW of 25 kDa nonspecifically recognized, corresponding to Der p 1. Moreover, product 2 showed the highest band intensity for group 2, followed by product 1. In the other products, the band intensity corresponding to 14 kDa was very weak.

MARIA[®] and ProteOn

As shown in Table 2, both quantitative methods expressing data in µg/ml showed the same tendency for the HDM major allergens; again, products 1 and 2 exhibited higher concentrations for each allergen, mainly in group 1, than the rest of products.

Amount of HDM major allergens in micrograms administered per day, week and month for each extract

Based on the daily maintenance dose recommended by each manufacturer in Spain, the amount of allergens administered was calculated in terms of daily, weekly and monthly doses.

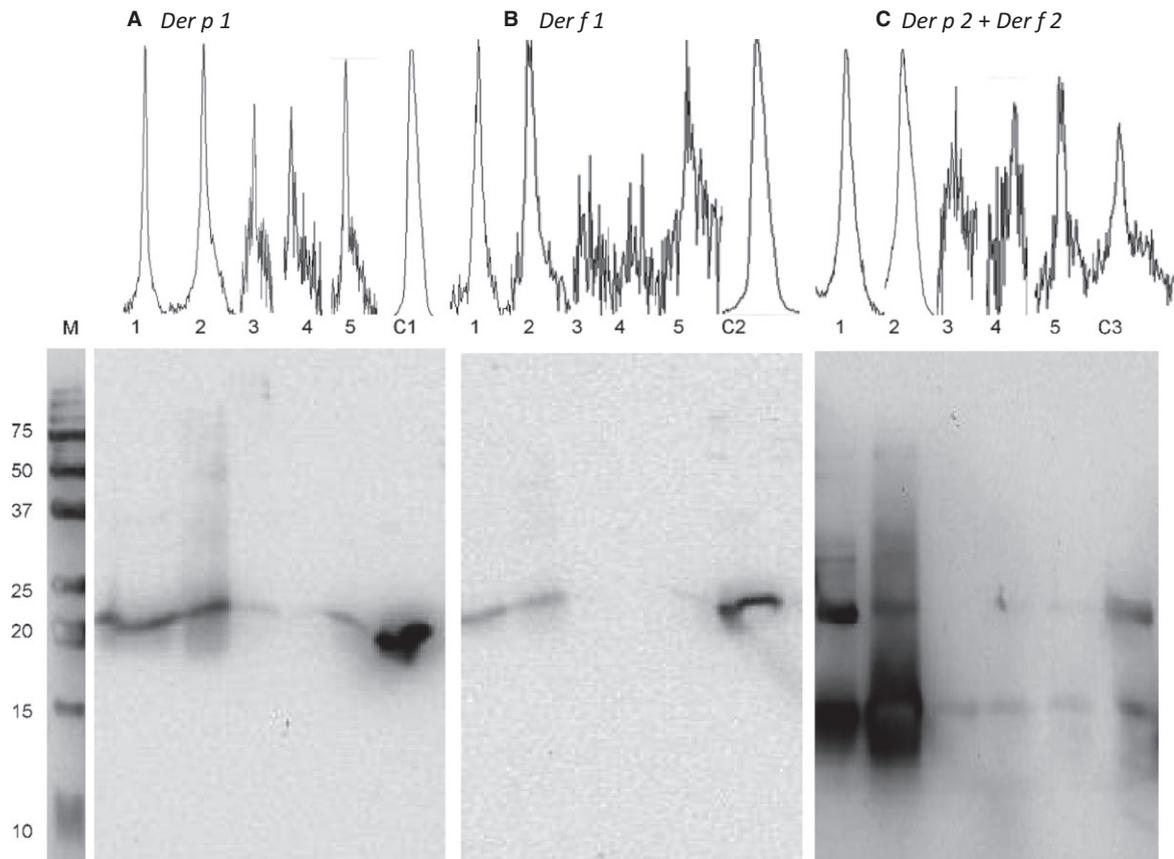


Figure 2 Immunoblotting. After transfer of proteins to PVDF membranes, they were faced against the following monoclonal antibodies: A) anti-Der p 1, B) anti-Der f 1, C) anti (Der p 2+ Der f 2). M = Marker MW. Blinded order: 1: Staloral 300 Rapid, 2: SLITone ULTRA, 3: TOL Forte, 4: Allergovac Sublingual Plus, 5:

Sublivac, 6: Control nDer p 1, 7: Control nDer f 1, 8: Control nDer p 2+ nDer f 2. On the top of gel, band intensity is shown for each sample present in gel, as measured in gels without signal saturation. Bands were quantified using Quantity One software.

Table 2 Measurements of allergen content with MARIA[®] and ProteOn

Content in µg/ml	MARIA Mean ± SD	ProteOn Mean ± SD
Der p 1		
Staloral 300 Rapid	12.4 ± 1.6	11.1 ± 1.0
SLITone ULTRA	14.5 ± 1.0	12.8 ± 0.8
TOL Forte	0.6 ± 0.3	0.5 ± 0.1
Allergovac Sublingual Plus	0.6 ± 0.1	2.5 ± 0.0
Sublivac	2.0 ± 1.4	1.1 ± 0.1
Der f 1		
Staloral 300 Rapid	12.4 ± 1.3	9.3 ± 0.7
SLITone ULTRA	6.2 ± 1.8	5.3 ± 1.9
TOL Forte	0.3 ± 0.3	0.9 ± 0.1
Allergovac Sublingual Plus	0.2 ± 0.1	1.0 ± 0.3
Sublivac	2.8 ± 1.7	4.4 ± 1.9
Der p 2+ Der f 2		
Staloral 300 Rapid	0.6 ± 0.4	3.4 ± 0.3
SLITone ULTRA	1.5 ± 0.2	5.8 ± 1.6
TOL Forte	0.1 ± 0.1	1.1 ± n/a
Allergovac Sublingual Plus	0.2 ± n/a	0.6 ± 0.8
Sublivac	0.2 ± 0.0	0.3 ± 0.3

n/a, not applicable; SD, standard deviation.

Products are displayed in blinded order (1: Staloral 300 Rapid, 2: SLITone Ultra, 3: TOL Forte, 4: Allergovac Sublingual Plus, 5: Sublivac).

The data obtained with the antibody Der p 2+ Der f 2 supplied by Indoor Biotechnologies gave results which were too low, far from the expected values in the controls used for the MARIA assay (approximately 10 times lower than expected).

Monthly dosage of Der p 1 was higher than 100 µg for products 1 and 2, whereas for products 4 and 5 it was not higher than 14 µg in any analysis, and product 3 did not reach 2 µg. Similar figures could be observed for Der f 1 and Der p 2+ Der f 2. (Table 3)

The affinity constant (KD) values for HDM major allergens in each extract are summarized in Table 4. A lower KD value indicates a higher affinity of the antibody for the epitope. Product 3 and product 4 showed a higher (and thus worse) affinity constant (KD) for Der p 1 and Der f 1 compared with products 1, 2 and 5, which are found in the same order observed for the control extracts. Regarding Der p 2+ Der f 2, similar KD values were found except for product 3 that had a higher (worse) KD.

Literature review

The literature search identified 55 reviews/meta-analyses (see Appendix S1). The review of these articles led to the identification of 30 clinical studies in which SLIT-HDM was used. A table in the Appendix S1 displays the differences concerning the allergen content of the HDM products used in each study. Substantial differences were found in the allergen content of the HDM products used in each study. In some studies, allergen content (9/30) and/or

biological units (3/30) are not recorded. Only 2 of the 5 products studied are referenced and some of the studies published data with product presentations and dosages that are not commercialized now in Spain. Among the articles presenting data on the allergen content (21/30), the cumulative weekly administered doses varied across trials, with the minimum value being 1 µg and the highest amount reaching 332 µg for Der p 1. In the majority of the studies (22/30), a 50/50 *D. pteronyssinus* and *D. farinae* mix was used. Reported data on efficacy were scarce for most of the five products. A detailed report of the literature review will be published separately.

Discussion

Our study was performed with the SLIT-HDM products from five of the most important manufacturers in Spain, and revealed large differences, not only for protein content but also for major allergens content. Results showed a very good internal consistency and were in line with the reviewed literature.

In the Western blot studies, we could observe that monoclonal antibody (Der p2+ Der f2), employed by the CREATE project, recognizes a few amount of group 1 allergens as shown in Fig. 2. Besides, the data obtained with the antibody Der p 2+ Der f 2 yielded results that were too low, far from the expected values in the controls used for the MARIA[®]. Different sequence polymorphisms of the major allergens can influence the quantification when using this MARIA[®] kit (18–20). This fact could be responsible for such a low recognition in our assays.

We should also consider that within the CREATE project (12), no consensus was achieved for any of the main allergens of HDM, even though Der p 2 reached a high reproducibility (21). Our native control extracts for group 1 showed a high reproducibility; however, that was not true for group 2. The consistency in the comparison is better when using the quantitative values for the major allergens in each extract calculated with both methods (MARIA[®]/ProteOn), especially when the removal of excipients with Vivaspin[®] 3.0K was made, and a good correlation is also observed with previous qualitative and quantitative values. Again, a difference between two of the products (Staloral 300 Rapid and SLITone Ultra), with values above 10 µg/ml for Der p 1, and the rest of the extracts (none of them exceeding 3 µg/ml) is confirmed.

The data obtained for group 2 should be interpreted cautiously because the controls used do not validate the results. Proportionally, we observed more Der p 2-f Der p 2 in SLITone Ultra (in agreement with the blot). For products 3, 4, and 5, the amount of group 2 was at the limit of quantification. However, the potency measurements in HDM extracts are based on the presence of the major allergen (in this case Der p 1+ Der f 1) because the role of group 2 is uncertain (11).

We have used the specific monoclonal antibody as a ligand to measure each one of the major allergens in HDM. There are very few studies that use this technique in allergy (22–26).

Table 3 Calculation of daily, weekly and monthly ordinary dosages (μg) according to the measurements made with MARIA and ProteOn

	Daily dosage ($\mu\text{g}/\text{day}$)				Weekly dosage ($\mu\text{g}/\text{week}$)				Monthly* dosage ($\mu\text{g}/\text{month}$)						
	Staloral 300 Rapid	SLITone ULTRA	Allergovac Sublingual Plus	TOL Forte	Staloral 300 Rapid	SLITone ULTRA	Allergovac Sublingual Plus	TOL Forte	Staloral 300 Rapid	SLITone ULTRA	Allergovac Sublingual Plus	TOL Forte	Subllivac		
Calculation of dosages according to determinations of MARIA															
Der p 1	9.95 \pm 1.26	7.23 \pm 0.48	0.06 \pm 0.03	0.15 \pm 0.02	0.43 \pm 0.3	29.84 \pm 3.78	50.63 \pm 3.34	0.40 \pm 0.23	0.74 \pm 0.11	3.01 \pm 2.08	129.5 \pm 16.4	219.7 \pm 14.5	1.73 \pm 1.0	3.2 \pm 0.5	13.7 \pm 9.0
Mean \pm SD															
Der f 1	9.93 \pm 1.06	3.11 \pm 0.91	0.03 \pm 0.03	0.05 \pm 0.02	0.61 \pm 0.36	29.78 \pm 3.19	21.77 \pm 6.38	0.23 \pm 0.20	0.24 \pm 0.08	4.27 \pm 2.49	129.3 \pm 13.9	94.5 \pm 27.71	0.98 \pm 0.8	1.0 \pm 0.4	18.5 \pm 10.79
Mean \pm SD															
Der p 2+	0.46 \pm 0.32	0.76 \pm 0.1	0.01 \pm 0.01	0.04 \pm n/a	0.04 \pm 0.01	1.39 \pm 0.95	5.34 \pm 0.72	0.05 \pm 0.04	0.18 \pm n/a	0.31 \pm 0.07	6.0 \pm 4.1	23.2 \pm 3.1	0.24 \pm 0.2	0.78 \pm n/a	1.36 \pm 0.32
Der f 2															
Mean \pm SD															
Calculation of the dosage according to determinations of ProteOn															
Der p 1	9.25 \pm 0.27	6.42 \pm 0.38	0.05 \pm 0.01	0.6 \pm 0.01	0.24 \pm 0.02	26.59 \pm 2.44	44.94 \pm 2.67	0.32 \pm 0.04	2.89 \pm 0.05	1.67 \pm 0.13	115.4 \pm 10.6	195.0 \pm 11.6	1.9 \pm 0.5	12.97 \pm 0.22	7.05 \pm 0.28
Mean \pm SD															
Der f 1	7.41 \pm 0.54	2.67 \pm 0.97	0.09 \pm 0.01	0.23 \pm 0.06	0.94 \pm 0.4	22.23 \pm 1.61	18.67 \pm 6.76	0.63 \pm 0.04	1.15 \pm 0.30	6.57 \pm 2.83	96.5 \pm 7.0	81.0 \pm 29.3	2.4 \pm 0.2	4.97 \pm 1.32	28.5 \pm 12.3
Mean \pm SD															
Der p 2+	2.74 \pm 0.20	2.91 \pm 0.79	0.10 \pm n/a	0.00 \pm 0.20	0.07 \pm 0.07	8.21 \pm 0.61	11.97 \pm 0.89	0.70 \pm n/a	0.71 \pm 0.98	0.47 \pm 0.48	35.6 \pm 2.6	88.4 \pm 24.1	3.0 \pm n/a	3.07 \pm n/a	2.02 \pm 2.12
Der f 2															
Mean \pm SD															

n/a, not applicable; SD, standard deviation;

Administered volumes according to index record: Extract 1: 800 $\mu\text{l}/\text{day}$, 2400 $\mu\text{l}/\text{week}$, 10 416 $\mu\text{l}/\text{month}$; Extract 2: 500 $\mu\text{l}/\text{day}$, 3500 $\mu\text{l}/\text{week}$, 15 190 $\mu\text{l}/\text{month}$; Extract 3: 95 $\mu\text{l}/\text{day}$, 665 $\mu\text{l}/\text{week}$, 2886.1 $\mu\text{l}/\text{month}$; Extract 4: 240 $\mu\text{l}/\text{day}$, 1200 $\mu\text{l}/\text{week}$, 5208 $\mu\text{l}/\text{month}$; Extract 5: 215 $\mu\text{l}/\text{day}$, 1505 $\mu\text{l}/\text{week}$, 6531.7 $\mu\text{l}/\text{month}$.

*A month has been equalled to 4.34 weeks.

Table 4 Affinity constants of Der p 1, Der f 1 and Der p 2+ Der f 2 for the different extracts

	Der p 1 KD (10^{-10} M)	Der f 1 KD (10^{-10} M)	Der p 2+ Der f 2 KD (10^{-10} M)
Staloral 300 Rapid (Mean \pm SD)	2.86 \pm 2.57	4.77 \pm 4.16	40.1 \pm n.a.
SLITone ULTRA (Mean \pm SD)	0.92 \pm 0.31	5.52 \pm 6.90	29.3 \pm n.a.
TOL Forte (Mean \pm SD)	37.5 \pm 3.75	537 \pm 753	3750 \pm n.a.
Allergovac Sublingual Plus (Mean \pm SD)	13.0 \pm 18.2	26.1 \pm 32.5	47.3 \pm n.a.
Subliva (Mean \pm SD)	4.57 \pm 4.60	36.1 \pm 8.98	11.2 \pm n.a.

SD, standard deviation.

Association (KA) and disassociation (KD) constants are shown in Appendix S1.

Larenas-Linnemann et al. (10) compared the protein content and the relative potency (ELISA) of various masked allergen extracts (among them *D. pteronyssinus*) for SLIT from four European manufacturers. The allergenic content range was 2.8–24.0 μ g/ml for Der p 1 and 0–7.7 μ g/ml for Der p 2.

Mosges et al. (27) compared the biological activity of two SLIT preparations to establish a 'conversion factor' between them. The biological activity was 22 times superior for one of them.

In the present study, the differences between the solutions with the highest (Staloral 300 Rapid and SLITone ULTRA) and lowest values amounted to a factor of more than 24 for Der p 1, more than 62 for Der f 1 and more than 7 for Der p 2+ Der f 2.

Other authors have also reported differences among HDM diagnostic products (15, 28).

Our results, based on a consistent methodology, allow reliable comparisons to be performed for the main extracts for SLIT treatment with marketed HDM allergens that we obtained in the same way our patients do. Only some of them showed a significant allergen content, which confirms the heterogeneity of the marketed products suggested in the literature. Besides, these are the only ones that are backed by publications reporting efficacy in clinical trials (29). This was also highlighted by Sander et al. (30) for SLIT based on grass preparations. They highlighted the need to take into account these differences when designing the treatment for patients allergic to grass pollen. We emphasize the same need when prescribing SLIT-HDM in patients allergic to mites.

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There are no universally accepted AIT dosage guidelines, except for grass pollen tablets, and the meagre reported data suggest that some dosages could have been insufficient (7, 31). Our review confirms a high heterogeneity in allergen content when reported, with values ranging from 1 μ g to 332 μ g for Der p 1 in calculated weekly doses.

In the current study, we compared the composition and main allergens amount of most relevant SLIT-HDM products in Spain. The methods endorsed by CREATE have been applied in a critical manner to study the characteristics of such extracts, with advantages and disadvantages of each technique being evaluated.

In contrast to previous composition studies (10, 15, 28, 30), products were purchased in a pharmacy so that they were provided in the same way patients receive them, and afterwards masking for blinding was applied. Our data indicate that two of the five studied extracts (Staloral 300 Rapid and SLITone ULTRA) are different from the rest. Interestingly, according to reported data, the scientific evidence currently available for most of them is scarce (29).

As we postulated and other authors have suggested, choice of a SLIT-HDM immunotherapy is now a difficult task. Clinicians need to rely on the evidence, and they have to know the quality assurance processes applied and the exact allergens content for each marketed product.

The data presented in this study highlight that not all SLIT products are equivalent, and for most of them, there is a large scope for improvement. Thinking of our patients, we must keep moving towards that goal in the coming years.

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Conflict of interests

The authors had no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Materials and Methods.

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